Bioequivalence Study with Two Naproxen Sodium Tablet Formulations in Healthy Subjects

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Abstract

The purpose of this study was to find out whether the bioavailability of a 550 mg naproxen sodium (CAS 22204-53-1) tablet (Sunprox, test) produced by Sunward Pharmaceutical Sdn Bhd was equivalent to that produced by the innovator. The pharmacokinetic parameters assessed in this study were area under the plasma concentration-time curve from time zero to 72 hours (AUC_t), area under the plasma concentration-time curve from time zero to infinity (AUC_{inf}), the peak plasma concentration of the drug (C_{max}), time needed to achieve the peak plasma concentration (t_{max}), and the elimination half life ($t_{1/2}$).

This was a randomized, single blind, two-period, cross-over study which included 26 healthy adult male and female subjects under fasting conditions. In each of the two study periods (separated by a washout of one week) single dose of test or reference drug was administered. Blood samples were taken up to 72 h post dose, the plasma was separated and the concentration of naproxen were determined by HPLC-UV method.

In this study, the mean AUC_t, AUC_{inf}, C_{max}, and $t_{1/2}$ of naproxen from the test drug were 936.11 µg.h.mL^{-1}, 977.03 µg.h.mL^{-1}, 76.55 µg/mL, and 15.11 h, respectively. The mean AUC_t, AUC_{inf}, C_{max}, and $t_{1/2}$ of naproxen from the reference drug were 969.77 µg.h.mL^{-1}, 1013.72 µg.h.mL^{-1}, 75.92 µg/mL, and 15.11 h, respectively. The median t_{max} of he test drug and reference drug were 3.0 h and 2.0 h, respectively. The geometric mean ratios (90% CI) of the test drug/ reference drug for naproxen were 96.46% (94.30 – 98.66%) for AUC_t, 96.33% (94.03 – 98.69%) for AUC_{inf}, and 100.37% (95.90 – 105.05%) for C_{max}.

Based on this study, it can be concluded that the two naproxen sodium tablets (test and drug reference drug) were bioequivalent in term of the rate and extent of absorption.

Keywords: Naproxen; Naproxen sodium; CAS 22204-53-1; CAS-26159-34-2; Sunprox; Bioavailability; Bioequivalence; Pharmacokinetic

Introduction

Bioavailability and bioequivalence of drug products have emerged as critical issues in pharmacy and medicine during the last three decades. Bioavailability is a pharmacokinetic term that describes the rate and extent to which the drug ingredient is absorbed from a drug product and becomes available in the systemic circulation. The area under the concentration versus time curve (AUC) serves as the extent of absorption, the time to reach the peak concentration (t_{max}) reflects the rate of absorption, while the peak concentration (C_{max}) reflects both the extent and the rate of absorption.

Naproxen is a propionic acid derivative related to the arylacetic acid group of nonsteroidal anti-inflammatory drugs. Naproxen (CAS 22204-53-1) and naproxen sodium (CAS-26159-34-2) are known chemically as (S)-6-methoxy-a-methyl-2-naphthaleneacetic acid and (S)-6-methoxy-α-methyl-2-naphthaleneacetic acid, sodium salt, respectively. Naproxen has a molecular weight of 230.26 and a molecular formula of $C_{14}H_{14}O_3$. Naproxen sodium has a molecular weight of 252.23 and a molecular formula of $C_{14}H_{13}NaO_3$. Naproxen is an odorless, white to off-white crystalline substance. It is lipid-soluble, practically insoluble in water at low pH and freely soluble in water at high pH. The octanol/water partition coefficient of naproxen at pH 7.4 is 1.6 to 1.8. Naproxen sodium is an odorless crystalline powder, white to creamy in color. It is soluble in methanol and water at neutral pH.

Naproxen and naproxen sodium have the following structures, respectively:
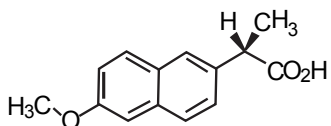

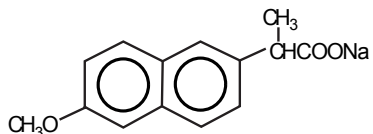Figure I: Structural formula of naproxen

Figure II: Structural formula of naproxen sodium

Naproxen is a nonsteroidal anti-inflammatory drug (NSAID) with analgesic and antipyretic properties. The sodium salt of naproxen has been developed as a more rapidly absorbed formulation of naproxen for use as an analgesic. Naproxen is indicated for the relief of the signs and symptoms of rheumatoid arthritis, osteoarthritis, ankylosing spondylitis, and juvenile arthritis. Naproxen is also indicated for the relief of the signs and symptoms of tendonitis, bursitis and acute gout; for the management of pain and primary dysmenorrhoea.

The mechanism of action of the naproxen anion, like that of other NSAIDs, is not completely understood but may be related to prostaglandin synthesis inhibition. NSAIDs inhibit both cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2) activities, and thereby inhibit synthesis of prostaglandins and thromboxane. The inhibition of COX-2 is thought to mediate the antipyretics, analgesic, and anti-inflammatory action of NSAIDs. Naproxen is rapidly and completely absorbed from the gastrointestinal tract with an in vivo bioavailability of 95%. The rapidity, but not the extent, of absorption is influenced by the presence of food

in the stomach. After administration of naproxen and naproxen sodium tablets, peak plasma levels are attained in 2 to 4 hours and 1 to 2 hours, respectively.

The plasma half-life of naproxen anion in human ranges from 12 to 17 hours (mean:14 hours). Steady-state levels of naproxen are reached in 4 to 5 days, and the degree of naproxen accumulation is consistent with this half-life. The corresponding half-lives of both naproxen's metabolites and conjugates are shorter than 12 hours, and their rates of excretion have been found to coincide closely with the rate of naproxen disappearance from plasma. Small amounts, 3% or less of the administered dose, are excreted in feces. In patients with renal failure, metabolites may accumulate.

The pharmacokinetic parameters of naproxen following an administration of 550 mg naproxen sodium tablet obtained from previous BE study based on literature[6] are 1202.6 ± 174.30 µg.h/mL for AUC_{0-t}, 1300.9 ± 204.20 µg.h/mL for $AUC_{0-inf,}$ 82.35 ± 12.48 µg/mL for C_{max} and 1.00 ± 0.71 h for t_{max}.

The most common adverse events reported after administration of naproxen are mild dyspepsia, gastric discomfort, constipation, diarrhoea, nausea, drowsiness, headache, dizziness, abdominal pain, sweating, itching, tinnitus, edema, and fatigue.

The objective of this study was to investigate the pharmacokinetic and bioavailability of two different oral naproxen sodium formulation following single dosing in healthy adult subjects in order to prove the bioequivalence between both preparation.

Subjects, Materials, Methods

Subjects and Study Design

The study was performed at PT. Equilab, Jakarta-Indonesia and was conducted according to the Declaration of Helsinki and the GCP, and GLP Guideline. The study protocol was reviewed and approved by the the committee of The Faculty of Medicine, University of Indonesia, Jakarta-Indonesia.

The test formulation was Sunprox (550 mg naproxen sodium equivalent to 500 mg naproxen), tablet (batch number 692-0807), manufactured by Sunward Pharmaceutical Sdn Bhd. Johor, Malaysia. The reference formulation was Synflex® (550 mg naproxen sodium equivalent to 500 mg naproxen) tablet, produced by Roche Pharma S.A. Spain was purchased at a local pharmacy in Malysia.

Twenty six (26) healthy subjects, 22 males and 4 females, aged between 19 and 46 years, body weight within normal

range (BMI= 18.13 – 22.97 kg/m^2), blood pressure within normal range (100 – 125 mmHg for systolic, and 60 – 80 mmHg for diastolic), pulse rate between 60 and 90 bpm, that had signed the informed consent were included in this study.

At least one week before and during the study period, the subjects were not allowed to take any drug including food supplement and herbal medicine. Pregnant women, nursing mothers, women childbearing potential without adequate contraception, subjects with known contraindications or hypersensitivity to naproxen, chronic gastrointestinal problems, liver dysfunction, clinically significant hematology abnormalities, renal insufficiency, and positive test results for HBsAg, anti-HCV, and/or anti -HIV , any surgical or medical condition (present or history) which might significantly alter the absorption, distribution, metabolism or excretion of the study drug, e.g. gastrointestinal disease including gastric or duodenal ulcers or history of gastric surgery, a donation or loss of 500 mL (or more) of blood within 3 months before this study's first dosing day, history of drug or alcohol abuse within 12 months prior to this screening, participation in a previous study within 3 months of this study's first dosing day were excluded from the study, as assessed of physical examination, vital signs (blood pressure, pulse/heart rate, respiratory rate and temperature), and laboratory values of liver function (AP, ALT, AST and total/direct bilirubin); renal function (serum creatinine and ureum); routine hematology (haemoglobin, leucocyte count, platelet and leucocyte differential count); blood glucose; routine urinalysis (pH, glucose, protein, and urine sediment), and immunology test for HBsAg, anti-HCV, and anti-HIV within 14 days prior to their first dosing day.

Treatment Phase and Blood Sampling

Subjects attended to PT Equilab International a night before drug administration and they were requested to fast from any food and drink except mineral water from 21:00 PM. In the morning (approximately 06:00 AM) of the dosing day (day 1), after an overnight fast, a pre-dose pharmacokinetic blood sample was taken. Then the study drug (one tablet of Sunward 550 mg naproxen sodium (Sunprox) or one tablet of Synflex®) were given at 07.00 AM with 200 mL of water and swallowed without chewing the drug in sitting posture. Subjects remained seated for 2 hours after drug administration, and avoiding severe physical exertion.

The venous blood samples were withdrawn 10 mL immediately before taking the drug (control), and 5 ml each at 15 and 30 minutes, and 1, 1.5, 2, 2.5, 3, 4, 6, 8, 12, 24, 36, 48, and 72 hours after drug administration. One week after the first drug administration (washout period), the same procedure was repeated the alternate drug.

The date and the time of taking each sample were recorded in the CRF. Lunch and dinner were provided 4 hours and 10 hours after drug administration.

The amount of food and water intake and physical activity for each individual subject were standardized during the sampling days. Xanthine-containing food or beverages and fruit juices were not allowed for 24 hours before and during the entire sampling days.

The subjects were under direct medical supervision during the days of naproxen sodium administration. Blood pressure, heart rate and adverse events were monitored during the blood sampling and also on follow-up study.

One physician and two nurses with sufficient qualifications and training were present at dosing time and stayed at the site until the last subject left the study unit; thereafter they were reachable by mobile telephone.

Assay Methodology

Method of Analysis

The naproxen concentrations in plasma were assayed using a fully validated high performance liquid chromatography with ultra violet detection (HPLC-UV) method, with respect to adequate sensitivity, specificity, linearity, recovery, accuracy and precision (both within and between days). The Stability of the samples under frozen conditions, at room temperature, and during freeze-thaw cycle were also determined. The following data were taken from the validation report: Calibration curve for naproxen ranged from 0.50 to 100.41 μg/mL; linear relationship between concentration and signal intensity were obtained (r = 0.9999); the limit of quantitation (LOQ) was 0,50 μg/mL; precision: intra-assay coefficient of variation were 2.06%, 0.73%, and 1.44% at low (1.51 μg/mL), medium (25.10 μg/mL), and high (75.31 μg/mL) concentrations, respectively; inter-assay coefficient of variations were 4.59%, 6.12%, and 3.40% at low, medium, and high concentrations, respectively; *accuracy:* intra assay (% diff) were ranged from -7.33% to -3.27% for the low concentrations, +12.12% to +14.38% for the medium concentrations, and from +1.26% to +5.35% for the high concentrations; inter assay (% diff) were ranged from -13.58% to +3.42% for the low concentrations, -3.46% to +14.38% for the medium concentrations, and from -1.84% to +9.14% for the high concentrations.

Assay procedure

The procedure was applied for the extraction of subject

samples, calibration and quality control standards. Plasma sample was dispensed in an appropriate tube, and an appropriate solvent was added. The content of the tube was vortexed and centrifuged. The organic phase was transferred to a vial and an aliquot was injected into the HPLC-UV at λ 240 nm system with a suitable condition. Calibration standards, controls, and samples were processed in batches.

Instrument and analysis conditions

A liquid chromatography with LiChroCART[(R)] 125-4 Lichrospher[(R)] 100 RP-18 e (5 µm) (Waters, 2795) and pre column of Symmetry C18 5µm, 3.9 x 20mm were used for separation of naproxen. The mobile phase was acetonitrile : KH_2PO_4 25 mM pH 3,00 (45 : 55, v/v). The flow rate was 1.0 mL/min and the injection volume was 20 µL. Detection were performed with ultra violet at 240 nm.

Pharmacokinetic Evaluation

The non-compartmental pharmacokinetic analysis method was employed to determine the pharmacokinetic parameter of naproxen. C_{max} and t_{max} were obtained directly from the observed data. The AUC_t is calculated by the trapezoidal method. The AUC_{inf} was calculated as $AUC_t + C_t/k_e$, where C_t was the last quantifiable concentration, k_e was the terminal elimination rate constant and was determined by least-squares regression analysis during the terminal log-linear phase of the concentration–time curve. The $t_{1/2}$ was calculated as $0.693/k_e$.

Statistical Evaluation

EquivTest version 2.0 (Statistical Solution Ltd., Saugus, MA, USA) was used to perform the statistical analyses of AUC_t, AUC_{inf}, and C_{max} using analysis of variance (ANOVA) after transformation of the data to their logarithmic (ln) values. Using the error variance (S^2) obtained from the ANOVA, the 90% confidence intervals (CIs) were calculated from the following equation:

$$90\% \text{ CI } = \left(\overline{X}_T - \overline{X}_R \right) \pm t_{0.1(v)} \sqrt{s^2 \times \frac{2}{n}}$$

- $\overline{X}_T, \overline{X}_R$: the means of the ln transformed values for the test product (T) and the reference product (R)

- S^2 : the error variance obtained from the ANOVA
- n : the number of subjects
- $t_{0.1}$: the t value for 90% CI
- v : the degree of freedom of the error variance from the ANOVA

The anti ln of the above confidence intervals were the 90% CIs of the ratios of the test / the reference geometric means. The power of study would be 90 % with 0.05 alpha. The acceptance criteria for bioequivalence were that the 90% CIs of the geometric mean ratios 0.80 − 1.25 for the AUC and Cmax. The tmax difference was analyzed non-parametrically on the original data using Wilcoxon matched-pairs test.

Result

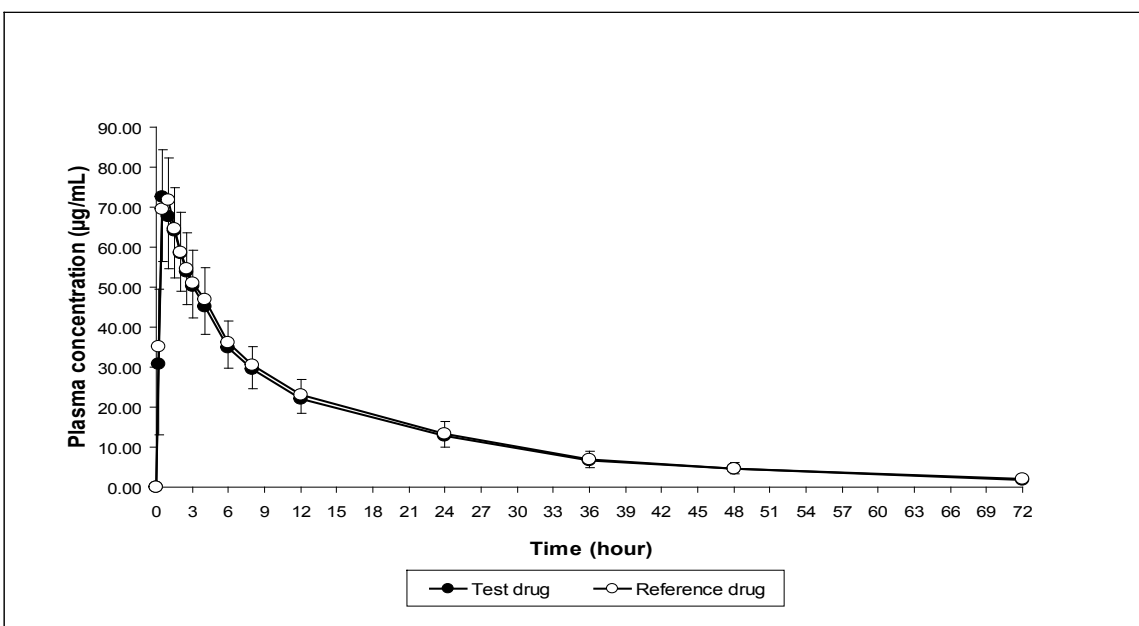A total 26 subjects were invited to participate in this

Figure 1: Mean plasma concentration- time profiles of naproxen in healthy subjects (n=24) after oral administration of 550 mg naproen sodium tablet (test) and that produced by the innovator (reference).

study (Gender and age on what basis-reference). Two subjects were dropped out from the study, because they did not follow the study completely until the last blood sampling because of personal reason. Twenty four subjects only were analyzed for pharmacokinetic evaluation. There was no adverse event encountered during the study. Mean plasma concentrations versus time profiles of naproxen after administration of both formulation in healthy subjects (n=24) are shown in Fig.1. The mean plasma concentration-time curve of the test product and the reference drug were comparable.

In this study, the mean (SD) AUC_t, AUC_{inf}, C_{max}, and $t_{1/2}$ of naproxen from the test drug were 936.11 (159.98) µg.h.mL^{-1}, 977.03 (173.40) µg.h.mL^{-1}, 76.55 (13.98) µg/mL, and 15.11 (1.11) h, respectively, with the median (range) t_{max} of 0.50 (0.50 – 1.50) h. The mean (SD) AUC_t, AUC_{inf}, C_{max}, and $t_{1/2}$ of naproxen from the reference drug were 969.77 (169.56) µg.h.mL^{-1}, 1013.72 (186.26) µg.h.mL^{-1}, 75.92 (11.18) µg/mL, and 15.11 (1.40) h, respectively, with the median (range) t_{max} of 1.00 (0.50 – 2.00) h. The pharmacokinetic parameters AUC_t, AUC_{inf}, C_{max}, and t_{max} were used for bioequivalence evaluation. The reference drug are presented in Table 1.

AUC_t, AUC_{inf} and C_{max} of test drug and the reference drug are presented in Table 1. The 90% confidence intervals for geometric mean ratios of test/reference for AUC_t, AUC_{inf} and C_{max} were within the acceptable limits (80 -125%) of bioequivalence which implies that the bioequivalence criteria were met. Evaluation of original data with Wilcoxon matched-pairs test showed that was no statistically significant difference between the two formulations for t_{max} values.

Discussion

The aim of the present randomized, single-blind, two-period, cross-over study with one-week wash-out period was to compare the bioavailability of the test naproxen sodium tablet produced by Sunward Pharmaceutical Sdn Bhd (Sunprox 550) with the reference naproxen sodium tablet (Synflex®). The formulations were administered to overnight fasted in order to eleiminate the influence of food on drug absorption.

For bioequivalence study, AUC_t, C_{max} and t_{max} were the main target parameters in order to assess possible bioequivalence between both preparations. Based on bioequivalence guideline, the acceptance criteria for bioequivalence were that the 90% confidence intervals of the the test/reference geometric means ratio for both compounds were in the range 0.80 – 1.25 for the AUC and C_{max}.

The result of this study showed that the 90% confidence intervals of the test/reference AUC ratio and C_{max} ratio were within the acceptable range for bioequivalence.

The mean (SD) elimination half-lives ($t_{1/2}$) of naproxen for the test drug was 15.11 (0.11) h and for the reference drug was 15.11 (0.40) h. These values were within the naproxen half-life range based on the literature, which is 12 – 17 h (mean: 14 hours). In each subject, the AUC_t value of naproxen was more than 80% compared to the value of AUC_{inf} (% AUC_t / AUC_{inf} ratio > 80%, 95.94% for test drug and 95.82% for reference drug), indicating that the sampling time was sufficiently long to ensure an adequate description of the absorption phase.

The median (range) of the time to reach maximum naproxen plasma concentration (t_{max}) of the test drug was 0.50 (0.50 – 1.50) h and 1.00 (0.50 – 2.00) h for the reference drug. Using Wilcoxon matched-pairs test on the original data, the difference between the two drugs (test and reference drug) t_{max} values were not significantly different. These

Parameter	Test Drug	Reference Drug	Mean Ratio (90% CI)	%CV	Wilcoxon matched-pairs test
- AUC_t (µg.h/mL)	936.11 ± 159.98	969.77 ± 169.56	96.46% (94.30 – 98.66%))	4.47 %	-
- AUC_{inf} (µg.h/mL)	977.03 ± 173.40	1013.72 ± 186.26	96.33% (94.03 – 98.69%)	4.80%	-
- C_{max} (µg/mL)	76.55 ± 13.98	75.92 ± 11.18	100.37% (95.90 – 105.05%)	9.17%	-
- t_{max} (h)	0.50 (0.50 – 1.50)	1.00 (0.50 – 2.00)	-		NS
- $t_{1/2}$ (h)	15.11 ± 1.11	15.11 ± 1.40	-		NS

Table 1: Pharmacokinetic parameters and statistical comparison of naproxen after 550 mg naproxen sodium single dose administration of test and reference drug in 24 healthy subjects.

values were also within the naproxen t_{max} range in the literature, which is 1 to 2 hours after administration of naproxen sodium tablets.

In the present study, the intra subject coefficient of variance (% CV) obtained from the ANOVA for naproxen was 4.47%, it means that the study only required a sample size of less than 24 subjects. Therefore this study had an adequate power to confirm a statistical conclusion.

During this study, two subjects (subject 25 and 26) were dropped out at period 2, because they did not follow the study completely until the last blood sampling because of personal reason. There was no adverse event encountered and no protocol deviation during the study.

Conclusion

Based on the pharmacokinetic and statistical result of this study results, it can be concluded that the two of 550 mg naproxen sodium (test and reference drug) were bioequivalent in term of the rate and the extent of absorption.

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